Proc. Natl. Acad. Sci. USA
Vol. 74, No. 9, pp. 4025–4028, September 1977
Medical Sciences

Amyloid protein SAA is associated with high density lipoprotein from human serum

(apolipoproteins)

EARL P. BENDITT AND NILS ERIKSEN

Department of Pathology SM-30, School of Medicine, University of Washington, Seattle, Washington 98195

Contributed by Earl P. Benditt, June 21, 1977

ABSTRACT **Human serums contain a protein antigenically related to protein AA, the principal protein of a major class of amyloid substance. The serum antigen, SAA, occurs mainly in a high molecular weight form, 1 to 2 × 10^5. This work shows that the bulk of the SAA sediments at density 1.12 g/cm^3 and floats at density 1.21 g/cm^3, as does the high density lipoprotein HDL_3. SAA is associated with the apolipoproteins ApoA-I and ApoA-II. The total cholesterol:total protein ratio of the fraction with density 1.12–1.21 g/cm^3 is 0.2:1, consistent with that of HDL_3. Acid treatment dissociates a significant portion of the SAA constituent of the HDL_3 fraction into low molecular weight species of the order of 13,000. The quantity of SAA may vary from 0.1% up to 1% or more of the total protein of HDL_3.**

Amyloidosis is the term given to a group of diseases characterized by extracellular deposits in various tissues of a material, amyloid substance, now known to be comprised largely of protein. Chemically distinct varieties of amyloid deposits are known (1). Recently most emphasis has been placed upon those cases in which the amyloid substance has a significant complement of immunoglobulin light chain fragments (2). However, the first protein extracted from amyloid substance and recognized as a major constituent of one group of amyloid substance was the polypeptide now called amyloid protein AA (3). This protein was found in the amyloid deposits associated with various chronic inflammatory diseases, such as rheumatoid arthritis, ankylosing spondylitis, chronic osteomeylitis, and tuberculosis in humans. All amyloid deposits of animals so far examined, including monkey, mink, mouse, guinea pig, and duck, yield upon extraction a polypeptide homologous by amino acid sequence with the human protein AA (4–7). Human and monkey protein AA are most nearly alike and consist of 76 amino acid residues, of molecular weights about 8500 and 8620, respectively (8, 9). The amino acid sequence of protein AA is not homologous with any known immunoglobulin or with any protein so far identified.

Antibodies raised in rabbits against protein AA derived from human amyloid tissue deposits react with a serum constituent called SAA (10–12). The immunoreactive protein SAA has been variously reported to have a molecular weight ranging from 80,000 to 200,000, as estimated by gel chromatography or electrophoresis (10–13). Treatment of serum containing macromolecular SAA with acid causes partial degradation of SAA to a peptide with an estimated molecular weight of about 11 to 15 × 10^3 (13–15); this protein has an NH_2-terminal amino acid sequence identical with that of tissue protein AA (14, 15). It is thought that the low molecular weight peptide is derived from the "native" macromolecular SAA and that the low molecular weight SAA component is the precursor of the protein AA of tissue amyloid deposits.

The native state of SAA and its physiological functions have not yet been described. Recently, a model for the apolipoproteins accounting for their combined hydrophobic and hydrophilic (i.e., amphipathic) character has been proposed (16). In a search for protein sequences having these amphipathic properties, it was found that protein AA has such sequence characteristics (17). This similarity led us to inquire whether or not SAA is part of the lipoprotein complex. In what follows we present evidence that by the usual operational criteria the antigenically identified amyloid-related serum protein is associated with the serum high density lipoproteins floating in the range of HDL_3 (1.12–1.21 g/cm^3) and appears to be a part of the HDL_3 apolipoprotein complex.

METHODS AND MATERIALS

Human protein AA was isolated from liver tissue obtained at autopsy from a patient with pulmonary tuberculosis, according to methods previously described (5). The initial product was further purified by rechromatography through Sephadex G-100 in 6 M urea/0.2 M NaCl/formic acid at pH 3.0.

Antiserums to protein AA were obtained by intradermal or intramuscular injection of rabbits with 0.4–0.5 mg of the protein dissolved at pH 10 in 0.5 ml of dilute NaOH and emulsified with an equal volume of complete Freund's adjuvant after the solution had been brought to incipient precipitation (pH 8) with traces of 5 M HCl. Two weeks after the initial injection the rabbits were given an intravenous booster injection of 0.5 mg of antigen without the adjuvant; fortnightly spaced local and intravenous booster injections were repeated at 6 weeks and 4 months. One year after the initial injection the local and intravenous injection sequence was repeated; serum obtained 1 week after the final booster injection had a high antibody level and was used for immunoassay.

Double immunodiffusion tests were done in Hyland Immuno-Plates (Travenol Laboratories), using the antiserum concentrated 3-fold by vacuum dialysis against pH 7.4 phosphate-buffered saline. When the difficultly soluble protein AA was used as antigen, it was dissolved in dilute NaOH at pH 9–9.5; other antigens were either human serum or serum fractions in phosphate-buffered saline.

Radiolabeling of protein AA with ^{125}I was done enzymatically with immobilized lactoperoxidase by an adaptation of the method of Marchalonis (18). In a typical procedure the protein AA (200 μg), dissolved in 35 μl of 1% sodium dodecyl sulfate ($NaDodSO_4$)/50 mM phosphate at pH 7.5, was mixed with the pellet obtained by centrifugation of 10–15 μl of a suspension of immobilized lactoperoxidase (Worthington) in a polyeth-

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: AA, tissue-derived nonimmunoglobulin amyloid protein; SAA, serum component related to AA; $NaDodSO_4$, sodium dodecyl sulfate; Tween 20, polyoxyethylene sorbitan monolaurate; HDL_3, high density lipoprotein isolated at solvent density 1.125–1.21 g/cm^3; ApoHDL, delipidated HDL.

ylene centrifuge tube. Two hundred microcuries of carrier-free $Na^{125}I$ (Amersham) in 2 μl of dilute NaOH (pH 8–11) and 1 μl of a $1/1000$ dilution of 3% H_2O_2 in 0.05 M phosphate (pH 7.5) were added to the tube. The reaction was allowed to proceed over a period of 3 min with intermittent mixing on a Vortex; then it was terminated by the addition of 100 μl of 0.05% NaN_3 in 0.05 M phosphate (pH 7.5). The mixture was centrifuged for 1 min in a Beckman 152 Microfuge, the supernate was withdrawn, and the pellet was washed by centrifugation with 100 μl of 1% $NaDodSO_4$/50 mM phosphate at pH 7.5. The combined supernate and washing were passed through a 0.9 × 58-cm column of Sephadex G-50 equilibrated against 0.01% $NaDodSO_4$/10 mM phosphate at pH 8.6. The protein AA emerged in coincidence with a peak of radioactivity. The two effluent fractions with the highest ultraviolet absorbance and radioactivity were combined; the pool had an estimated specific activity of 6×10^8 dpm per mg of protein AA.

Radioimmunoassay for SAA was done by a micromodification of the two-antibody system (19). Ten microliters of a $1/10$ or $1/20$ dilution (in phosphate-buffered saline) of antiserum to protein AA, or similarly diluted normal rabbit serum for control, was introduced into a 400-μl polyethylene tube with attached cap. Human serum (2–50 μl, undiluted, diluted, or fractionated) to be tested for SAA activity was added, followed by 10 μl of a suitable dilution of stock ^{125}I-labeled protein AA. The diluent for the labeled protein AA was 0.01% $NaDodSO_4$/10 mM phosphate at pH 7.4, and included sufficient polyoxyethylene sorbitan monolaurate (Tween 20) to give a concentration of 0.04–0.07% (vol/vol) in the final reaction mixture. The Tween 20 was effective in keeping the level of nonspecific radioactivity at 2% or less of the radioactivity initially added (20). After mixing of the reactants, the tubes were momentarily centrifuged to consolidate isolated droplets, then incubated for 1 hr at 37° and 30–60 min at room temperature, or alternatively, overnight at 4°. After this primary incubation, an amount of goat antiserum to rabbit IgG (Miles-Yeda) previously determined to give maximal precipitation was added (5–20 μl per tube, depending on amount of rabbit serum present and titer of anti-IgG serum). After mixing and momentary centrifugation, the total radioactivity in each tube was measured in a Nuclear-Chicago automatic gamma counter. One hour after addition of the second antibody, 100 μl of wash solution (equal volumes of phosphate-buffered saline and 1% Tween 20/0.01% $NaDodSO_4$/10 mM phosphate at pH 7.4) was added to each tube. After brief mixing, the tubes were centrifuged for 2.5 min in the Microfuge (13,800 × g, maximum) and the supernates were removed by aspiration. The pellets were thoroughly dispersed in 100-μl volumes of wash solution by Vortex mixing, the tubes were centrifuged, and the supernates were aspirated as before. As a final rinse, 200 μl of wash solution was added to each tube, and the tubes were centrifuged for 1 min to repack the precipitate. The bottom of the tube with precipitate was then cut off just above the top of the precipitate and the tube bottom with the precipitate was transferred to a tube in which radioactivity was measured. Net radioactivity in the precipitate was obtained by subtracting the radioactivity in the normal rabbit serum control.

Several concentrations of nonradioactive protein AA included in each assay series were used to construct standard displacement curves. From the standard displacement curves the amount of AA equivalent protein was estimated. The sensitivity of the method is of the order of 1–2 ng of AA equivalent. At 50% inhibition of binding of radioactive AA, the ratio of nonradioactive AA to radioactive AA was approximately 0.7, indicating good retention of protein AA antigenic determinants in the labeled material.

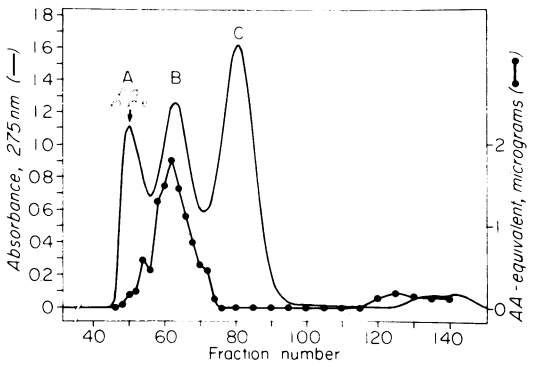

FIG. 1. Sephadex G-200 chromatography of human serum (2.5 ml) obtained after typhoid immunization. Solvent, phosphate-buffered saline; flow rate, 19 ml/hr; fraction volume, 3.7 ml.

Centrifugal flotation was done at 40,000 rpm (105,000 × g) in a Beckman 40 rotor and L2-65B preparative ultracentrifuge. Solvent densities were adjusted by the addition of solid KBr to serum or infranates according to density tables based on handbook values for the composition of aqueous KBr solutions. Dilutions of serum or infranates to volumes convenient for loading 10 ml per tube were made with KBr solutions of appropriate density. Specific gravities were measured on selected samples and corresponded satisfactorily with expected values. After centrifugation for 44 hr at 5°, successive 2.5-ml layers were removed from the tubes by careful aspiration into a syringe fitted with a flat-tipped needle. Corresponding layers from replicate tubes were pooled and dialyzed against phosphate-buffered saline to remove KBr prior to SAA assays or gel chromatography; when necessary, pools were concentrated in collodion bags (Schleicher and Schuell) by vacuum dialysis against phosphate-buffered saline.

Gel chromatography of serum and centrifugal fractions was carried out in a 2.5 × 92-cm column of Sephadex G-200 by upward flow of phosphate-buffered saline at 4°.

Total protein was estimated with the Folin–Ciocalteu phenol reagent (21), with bovine serum albumin as standard.

Total cholesterol was measured enzymatically after hydrolysis of cholesterol esters to free cholesterol by cholesterol ester hydrolase (22).

EXPERIMENTAL OBSERVATIONS

The sedimentation characteristics of the principal components in human serum reacting with the antibody to purified protein AA derived from human tissues were investigated first on a pool of human serum with a high SAA titer (3000 ng of AA equivalent per ml of serum). These trials showed that the SAA sedimented at densities less than 1.12 g/cm^3 and a major portion (60%) floated at a density of 1.21 g/cm^3. In this serum pool, which had been stored for months at −20°, some material sedimented as though it were protein without lipid at a density of 1.25 g/cm^3.

On the basis of the preceding studies, a series of experiments was undertaken with freshly drawn serum obtained with informed consent from a woman 20 hr after a typhoid immunization injection, a procedure that elevates the SAA titer (23). Chromatography of a sample of this serum on Sephadex G-200 gave the protein pattern shown in Fig. 1. The bulk of the measurable SAA activity is seen to chromatograph in the component labeled B, in which immunoreactivity to IgG was contained. A very small peak of immunoreactive material is present in the low (10,000–15,000) molecular weight range.

The chromatographic pattern of the fraction of the serum floating at a density of 1.21 g/cm^3 after removal of the portion

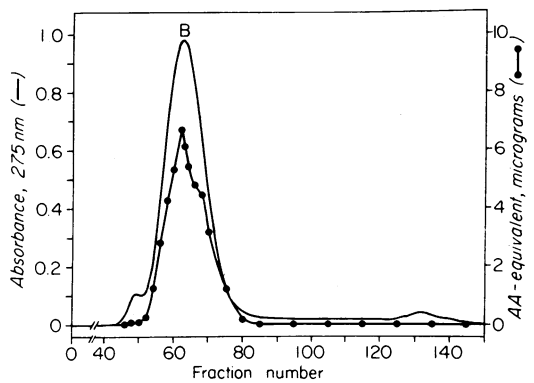

FIG. 2. Sephadex G-200 chromatography of HDL_3 fraction of human serum. Sample, 18 mg of total protein in 2.5 ml; other conditions as in Fig. 1.

floating at a density of 1.12 g/cm^3 is shown in Fig. 2. A small amount of material absorbing at 275 nm appeared at the front. However, the bulk of the material appeared in a single symmetrical peak corresponding to a molecular weight of around $1.6 \pm 0.3 \times 10^5$. This peak contained neither albumin nor IgG, as determined by immunodiffusion against potent antiserums to these proteins. The SAA activity was confined to this main chromatographic constituent, in which the estimated AA-equivalent was 10 μg/mg of total protein.

Assay of the fraction of serum floating at density 1.12 g/cm^3, containing the low density lipoprotein and high density lipoprotein of density 1.06–1.12 g/cm^3, showed only about 2% of the total SAA found by radioimmunoassay. The major HDL_3 fraction (density 1.21 g/cm^3, top layer after removal of material floating at density 1.12 g/cm^3) contained 57% of the SAA. A further 17% of the immunoreactive material was found in the second layer of the density 1.21 g/cm^3 fractionation, associated with a correspondingly diminished amount of HDL_3 identified by Sephadex G-200 chromatography. Altogether, SAA associated with the HDL_3 appeared to account for 74% of the total; 11% was present in the third layer and 15% in the bottom layer.

To further characterize the serum lipoprotein fraction containing SAA, we examined the chromatographically (Sephadex G-200) purified top layer peak fractions of density 1.12–1.21 g/cm^3 (apex, component B, Fig. 2) by immunodiffusion with specific antiserums to HDL_3 apoproteins ApoA-I and ApoA-II. A strong reaction with the antiserum to ApoA-I and a weaker, but definite, reaction with antiserum to ApoA-II were obtained, consistent with the relative amounts of the apoproteins in HDL_3 (24). The total cholesterol:total protein ratio in 15 pooled fractions from the chromatographic peak was estimated to be 0.2, consistent with recorded values (24).

Tests of the correspondence of material derived from tissues and in whole serum and the several fractions thereof were performed by immunodiffusion against the antiserum to AA; an example is shown in Fig. 3. The antigenic identity of the AA and the reacting constituent in the serum, in chromatographically separated high molecular weight SAA, in the HDL_3 fraction of the serum investigated here, and in an apolipoprotein preparation from a serum HDL_3 fraction of another individual is clearly evident.

In order to see if the low molecular weight serum AA constituent could be derived from the high molecular weight material we concentrated the ultracentrifugally prepared HDL_3 fraction after chromatographic purification by vacuum dialysis, brought it to 10% in formic acid, and incubated it for 24 hr at 37° to dissociate the SAA. The acid-treated concentrate was chromatographed on a Sephadex G-100 column (1.6 × 90 cm) with 10% formic acid as eluent. Of the total AA-equivalent material recovered, about 35% appeared in the low molecular weight region of 10,000–15,000 and about 60% appeared near the front of the chromatogram associated with unresolved larger molecular weight material, presumably apolipoprotein aggregates.

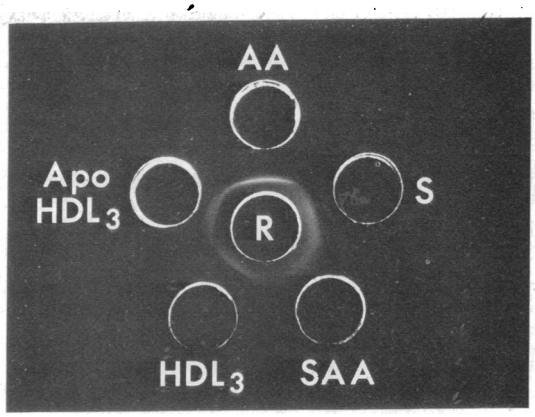

FIG. 3. Double immunodiffusion of rabbit antiserum (R) to human protein AA apposed to the immunogen (AA), serum from typhoid-stimulated donor (S), chromatographically separated serum amyloid component (SAA), flotationally separated serum component at solvent density 1.12–1.21 g/cm^3 (HDL_3), and delipidated component of human serum (unstimulated donor) separated at solvent density 1.1–1.21 g/cm^3 ($ApoHDL_3$).

DISCUSSION

The data provide evidence that the bulk of the amyloid protein AA-related serum constituent, SAA, is associated with the HDL_3 fraction of the serum lipoproteins. In the serum of individuals having elevated concentrations of SAA the amount may be of the order of 1% of the total HDL_3 proteins; the content of SAA in HDL_3 fractions from serum of persons with normal levels of SAA is about one-tenth of this, or about 0.1% of the total HDL_3 apoproteins. The small amounts of SAA present in the HDL_3 fraction probably account for the fact that protein SAA has not been observed previously in the search for the apoproteins of HDL_3.

The identification of SAA as a small and apparently variable constituent of the high density lipoproteins is consistent with the idea concerning the amphipathic character of protein AA (25). Furthermore, the association of SAA with the HDL_3 may explain some of the peculiar variations found in the immunoassay of SAA observed by others who have studied its levels in serum (13, 26). Similar variability in the immunoreactive characteristics of apoprotein A-I of HDL have been found; these differences in reactivity seem to depend upon the part of the protein that is exposed when it is in the native high density lipoprotein particle, and various manipulations have been introduced to unmask the antigenic features of apolipoproteins of high density lipoproteins in order to stabilize assays for the lipoproteins (27, 28).

A logical question is whether SAA could form a part of and be derived from one of the known apoproteins. The only likely precursor is ApoA-I. Examination of the known sequence (29) does not reveal a corresponding sequence of any significant degree of homology; this is not apparently the source.

Several obvious questions are raised by this finding of the bulk of the SAA in the HDL_3 fraction of human serum: (*i*) Does the SAA reside in a special HDL_3 fraction or appear along with ApoA-I and ApoA-II on the same lipoprotein particle? This question is susceptible to an answer. (*ii*) What is the function of this apparently variable element of the lipoprotein complex?

(*iii*) Where does it come from? (*iv*) How is it metabolized? The last question bears upon the formation of amyloid deposits, since it now seems reasonable to suppose that SAA is the precursor of the protein AA found in tissue amyloid deposits.

Assuming that the SAA of low molecular weight forms a variable part of the apoprotein complex of HDL_3, one can visualize a possible mechanism for formation of protein AA of 8500 molecular weight from its SAA precursor of about 13,000 molecular weight. Since protein SAA and protein AA have the same NH_2-terminal portion, the portion lost from the SAA to form AA must be a COOH-terminal fragment, molecular weight about 4500. If this COOH-terminal portion were more mobile and exposed on the HDL_3 lipid particle surface, then proteases could have access to this portion of SAA. Where the proteases come from and of what type they are remain to be ascertained. Deposition would require release of AA and the association of AA monomers into fibrils.

Currently there is great interest in the function of HDL as it relates to lipoprotein metabolism in artery wall cells (30–32). The observations presented here and the characteristic location of amyloid deposits containing protein AA in subendothelial locations, in small artery and in vein walls, suggest the possibility that endothelial cells and arterial wall smooth muscle cells are sites for some physiological operation of the SAA–HDL_3 complex.

We are grateful to Drs. J. J. Albers and William Hazzard of the University of Washington Department of Medicine for providing us with HDL_3 and ApoHDL_3 preparations and antiserums to ApoHDL preparations, to Dr. J. A. Glomset and staff of the University of Washington Regional Primate Research Center for helpful technical discussions, and to Mr. Ronald H. Hanson for skilled technical assistance. The work was supported by U.S. Public Health Service Grant HL-03174.

1. Benditt, E. P. & Eriksen, N. (1971) "Chemical classes of amyloid substance," *Am. J. Pathol.* **65**, 231–252.
2. Glenner, G. G., Terry, W. D. & Isersky, C. (1973) "Amyloidosis: Its nature and pathogenesis," *Semin. Hematol.* **10**, 65–86.
3. Cohen, A. S., Franklin, E. C., Glenner, G. G., Natvig, J. B., Osserman, E. F. & Wegelius, O. (1976) "Nomenclature," in *Amyloidosis*, eds. Wegelius, O. & Pasternack, A. (Academic Press, New York and London), p. ix.
4. Benditt, E. P. (1976) "The structure of amyloid protein AA and evidence for a transmissible factor in the origin of amyloidosis," in *Amyloidisis*, eds. Wegelius, O. & Pasternack, A. (Academic Press, New York and London), pp. 323–332.
5. Eriksen, N., Ericsson, L. H., Pearsall, N., Lagunoff, D. & Benditt, E. P. (1976) "Mouse amyloid protein AA: Homology with nonimmunoglobulin protein of human and monkey amyloid substance," *Proc. Natl. Acad. Sci. USA* **73**, 964–967.
6. Skinner, M., Cathcart, E. S., Cohen, A. S. & Benson, M. D. (1974) "Isolation and identification by sequence analysis of experimentally induced guinea pig amyloid fibrils," *J. Exp. Med.* **140**, 871–876.
7. Husby, G., Natvig, J. B., Sletten, K., Nordstoga, K. & Anders, R. F. (1975) "An experimental model in mink for studying the relation between amyloid fibril protein AA and the related serum protein, SAA," *Scand. J. Immunol.* **4**, 811–816.
8. Levin, M., Franklin, E. C., Frangione, B. & Pras, M. (1972) "The amino acid sequence of a major nonimmunoglobulin component of some amyloid fibrils," *J. Clin. Invest.* **51**, 2773–2776.
9. Hermodson, M. A., Kuhn, R. W., Walsh, K. A., Neurath, H., Eriksen, N. & Benditt, E. P. (1972) "Amino acid sequence of monkey amyloid protein A," *Biochemistry* **11**, 2934–2938.
10. Levin, M., Pras, M. & Franklin, E. C. (1973) "Immunologic studies of the major nonimmunoglobulin protein of amyloid. I. Identification and partial characterization of a related serum protein component," *J. Exp. Med.* **138**, 373–380.
11. Husby, G. & Natvig, J. B. (1974) "A serum component related to nonimmunoglobulin amyloid protein AS, a possible precursor of the fibrils," *J. Clin. Invest.* **53**, 1054–1061.
12. Benson, M. D., Skinner, M., Lian, J. & Cohen, A. S. (1975) " 'A' protein of amyloidosis. Isolation of a crossreacting component from serum by affinity chromatography," *Arthritis Rheum.* **18**, 315–322.
13. Ignaczak, T. F., Sipe, J. D., Linke, R. P. & Glenner, G. G. (1977) "Immunochemical studies on the nature of the serum component (SAA) related to secondary amyloidosis," *J. Lab. Clin. Med.* **89**, 1092–1104.
14. Anders, R. F., Natvig, J. B., Michaelsen, T. E. & Husby, G. (1975) "Isolation and characterization of amyloid-related serum protein SAA as a low molecular weight protein," *Scand. J. Immunol.* **4**, 397–401.
15. Rosenthal, C. J., Franklin, E. C., Frangione, B. & Greenspan, J. (1976) "Isolation and partial characterization of SAA—an amyloid-related protein from human serum," *J. Immunol.* **116**, 1415–1418.
16. Segrest, J. P., Jackson, R. L., Morrisett, J. D. & Gotto, A. M., Jr. (1974) "A molecular theory of lipid-protein interactions in the plasma lipoproteins," *FEBS Lett.* **38**, 247–253.
17. Segrest, J. P., Pownall, H. J., Jackson, R. L., Glenner, G. G. & Pollock, P. S. (1976) "Amyloid A: Amphipathic helixes and lipid binding," *Biochemistry* **15**, 3187–3191.
18. Marchalonis, J. J. (1969) "An enzymic method for the trace iodination of immunoglobulins and other proteins," *Biochem. J.* **113**, 299–305.
19. Morgan, C. R. & Lazarow, A. (1963) "Immunoassay of insulin: Two antibody system," *Diabetes* **12**, 115–126.
20. Etzrodt, H., Schleyer, M., Schröder, K.-E. & Pfeiffer, E. F. (1976) "The use of Tween 20 in the double antibody radioimmunoassay," *Z. Physiol. Chem.* **357**, 1205–1208.
21. Schacterle, G. R. & Pollack, R. L. (1973) "A simplified method for the quantitative assay of small amounts of protein in biologic material," *Anal. Biochem.* **51**, 654–655.
22. Allain, C. C., Poon, L. S., Chan, S. C. G., Richmond, W. & Fu, P. C. (1974) "Enzymatic determination of total serum cholesterol," *Clin. Chem.* **20**, 470–475.
23. Linder, E., Anders, R. F. & Natvig, J. B. (1976) "Connective tissue origin of the amyloid-related protein SAA," *J. Exp. Med.* **144**, 1336–1346.
24. Scanu, A. M. (1972) "Structural studies on serum lipoproteins," *Biochim. Biophys. Acta* **265**, 471–508.
25. Segrest, J. P. (1976) "Molecular packing of high density lipoproteins," *FEBS Lett.* **69**, 111–115.
26. Franklin, E. C. (1976) "Some properties of antisera to serum amyloid A protein (SAA): Inhibition of precipitation by complexing of SAA to albumin," *J. Exp. Med.* **144**, 1679–1682.
27. Schonfeld, G., Bradshaw, R. A. & Chen, J.-S. (1976) "Structure of high density lipoprotein: The immunologic reactivities of the COOH- and NH_2-terminal regions of apolipoprotein A-I," *J. Biol. Chem.* **251**, 3921–3926.
28. Shen, B. W., Scanu, A. M. & Kézdy, F. J. (1977) "Structure of human serum lipoproteins inferred from compositional analysis," *Proc. Natl. Acad. Sci. USA* **74**, 837–841.
29. Baker, H. N., Delahunty, T., Gotto, A. M., Jr. & Jackson, R. L. (1974) "The primary structure of high density apolipoprotein-glutamine-I," *Proc. Natl. Acad. Sci. USA* **71**, 3631–3634.
30. Stein, O. & Stein, Y. (1976) "High density lipoproteins reduce the uptake of low density lipoproteins by human endothelial cells in culture," *Biochim. Biophys. Acta* **431**, 363–368.
31. Weinstein, D. B., Carew, T. E. & Steinberg, D. (1976) "Uptake and degradation of low density lipoprotein by swine arterial smooth muscle cells with inhibition of cholesterol biosynthesis," *Biochim. Biophys. Acta* **424**, 404–421.
32. Fielding, C. J. & Havel, R. J. (1977) "Lipoprotein lipase," *Arch. Pathol. Lab. Med.* **101**, 225–229.